Yeast Artificial Chromosomes (YACs)

- Developed by Burke et al. (1987)

Cloning of Large Segments of Exogenous DNA into Yeast by Means of Artificial Chromosome Vectors

DAVID T. BURKE, GEORGES F. CARLE, MAYNARD V. OLSON

- Yeast-Based Cloning System (*Saccharomyces cerevisiae*)
- System Based on Ability to 'Harness' Cloned DNA with the Structural Elements Required for Propagation of a Linear Chromosome in Yeast
- Cloned Insert: ~100 to >1,000 kb, Linear DNA
- Spheroplast Transformation Procedure

  Technically Demanding
  Poorly Defined Upper Size Limit for Cloned Insert

- References

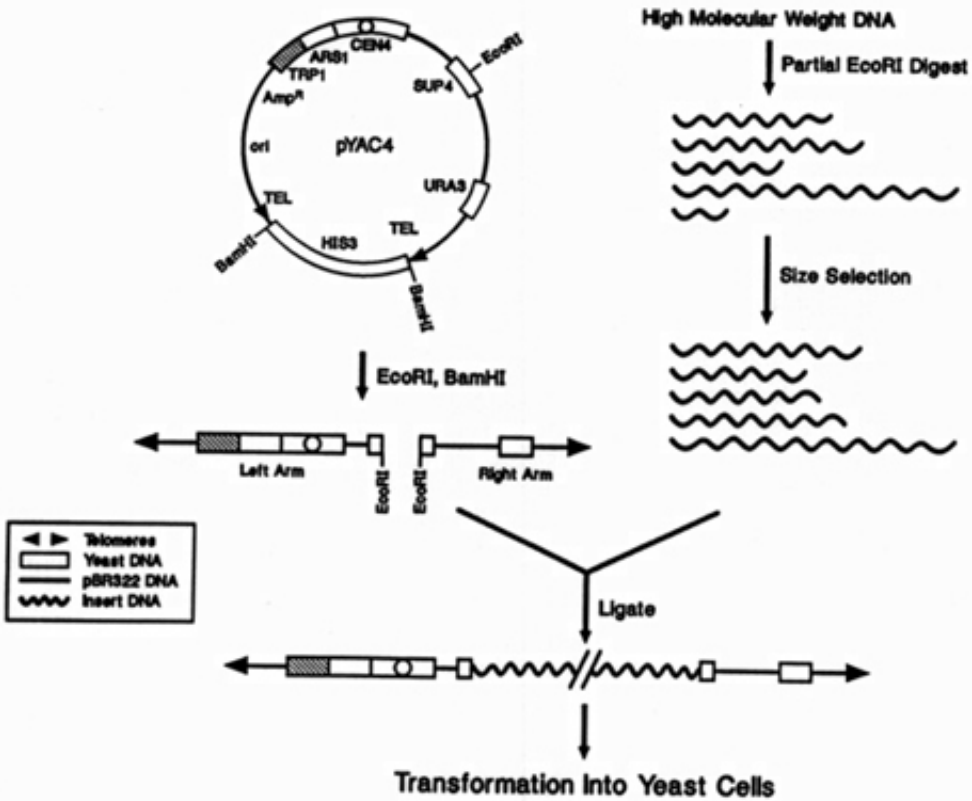
  Hieter et al. (1990), Schlessinger (1990), Burke (1991), Ramsay and Wicking (1991), Schlessinger and Kere (1992), Green et al. (1998)

YAC Cloning System

- YAC Vector

  2 Telomere Sequences
  1 Centromere Sequence
  1 ARS Sequence
  2 Yeast Selectable Markers (Not Antibiotic Selection)

- Strategy for YAC Cloning



Major Features of YACs

- Cloned DNA in Single Copy within Yeast Genome
  - Generally Same Structure and Size as Endogenous Chromosomes
  - Limited 'Access' to Cloned DNA (e.g., Gel Isolation)
- Chimerism as Major 'Problem'
  - Upwards of 40-60% of Clones in Total Mammalian DNA Libraries
  - Largely from 'Overly' Efficient Yeast Recombination System
    - [Green et al. (1991)]
- Instability (e.g., Internal Deletions) as More Minor 'Problem'
  - Difficult to Estimate Extent of Problem
  - Clearly Related to Recombination in Yeast
  - Might be Related to YAC Size
  - Influenced by Clone Handling
- Issues of Recombination-Deficient Host Strains
- Various Human, Mouse, and Rat Libraries Constructed

Human:

Washington University [Burke and Olson (1991), Brownstein et al. (1989)]
CEPH (Includes 'Mega-YACs') [Albertsen et al. (1990), Dausset et al. (1992)]
ICRF [Larin et al. (1991)]
ICI [Anand et al. (1989), Anand et al. (1990)]

Mouse:

Princeton [Burke et al. (1991), Rossi et al. (1992)]
St. Mary's [Chartier et al. (1992)]
ICRF [Larin et al. (1991, 1993)]
Whitehead [Kusumi et al. (1993), Haldi et al. (1996)]

Rat:

Harvard [Cai et al. (1997)]
Whitehead [Haldi et al. (1997, 1997)]

Strategies for Clone-Based Physical Mapping

- Two Key Components ('Jigsaw Puzzle Analogy')

  Cloned Fragments (Pieces of the Puzzle)
  Landmarks (Provide Clues for Aligning Pieces)

- Ultimately Want to Order Clones and/or Landmarks

- Ideally Want 'Access' to Both Clones and Landmarks

- Clone-Based Physical Mapping Typically Involves the Use of Landmarks to Assembly Clone 'Contigs'

  Contig: Overlapping Set of Clones that Together Contain a Contiguous Segment of the Source Genome

- Nature of Landmarks

  Must Provide 'Unique' Information About the DNA
  Must be Easy to Identify
  Can be Intrinsic or Extrinsic to the Clones

- Early Candidates for Landmarks: Restriction Sites (Intrinsic Landmarks)

Physical Maps of "Smaller" Genomes

- *E. Coli* [Kohara et al. (1987)]

The Physical Map of the Whole E. coli Chromosome: Application of a New Strategy for Rapid Analysis and Sorting of a Large Genomic Library

Yuji Kohara,* Kiyotaka Akiyama,* and Katsumi Isono†

- Yeast [Olson et al. (1986), Riles et al. (1993)]

Random-clone strategy for genomic restriction mapping in yeast

(DNA/chromosomes/λ cloning/algorithm)

Maynard V. Olson, James E. Dutchik, Madge Y. Graham, Garrett M. Brodeur, Cynthia Helms, Mark Frank, Mia MacCollin, Robert Scheinman, and Thomas Frank

Physical Maps of the Six Smallest Chromosomes of *Saccharomyces cerevisiae* at a Resolution of 2.6 Kilobase Pairs

Linda Riles,* James E. Dutchik,†,1 Amara Baktha,* Brigid K. McCauley,*,2 Edward C. Thayer,*,3 Mary P. Leckie,†,4 Valerie V. Braden,* Julie E. Depke†,5 and Maynard V. Olson†,6

- Nematode [Coulson et al. (1986)]

Toward a physical map of the genome of the nematode *Caenorhabditis elegans*

(ordered clone bank/genomic data base/clone matching)

Alan Coulson, John Sulston, Sydney Brenner, and Jonathan Karn

Early Physical Mapping of Human Chromosomes

- Strategies Analogous to those Used with *E. coli*, Yeast, and Nematode Applied to Several Human Chromosomes

  Cosmid Clones (e.g., Flow-Sorted Libraries)
  Restriction Map Construction and/or Fingerprint Analysis
  [e.g., Stallings et al. (1990)]
  Difficult to Construct Long-Range Contiguous Maps

- Shift in Strategies with the Development of YACs

- Modified Fingerprint-Based Strategies Attempted with YACs

- Distinguishing Features of YACs

  No Ability to Readily Purify Cloned DNA
  Fingerprint Analysis:
  1. Typically Requires Gel-Transfer Hybridization
  2. Typically Uses Repetitive Element-Specific Probe(s)

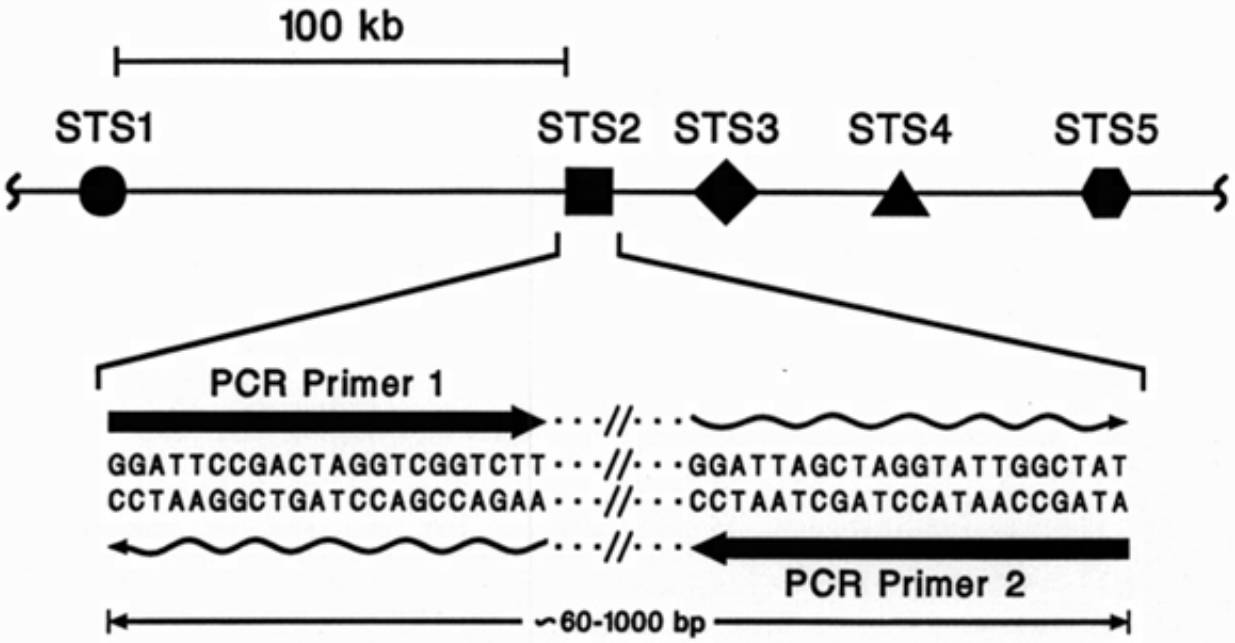
  Establish YAC 'Fingerprint' → Infer Overlap(s) with Other YACs

- Fingerprint-Based Approaches Provide Clone-Based Maps Without Deriving Extrinsic Landmarks

- Major 'Evolution' in Strategy Occurred with Development of PCR

Sequence-Tagged Sites (STSs)

- Development of PCR → Profound Impact on Physical Mapping
- STSs as Landmarks for Constructing Physical Maps

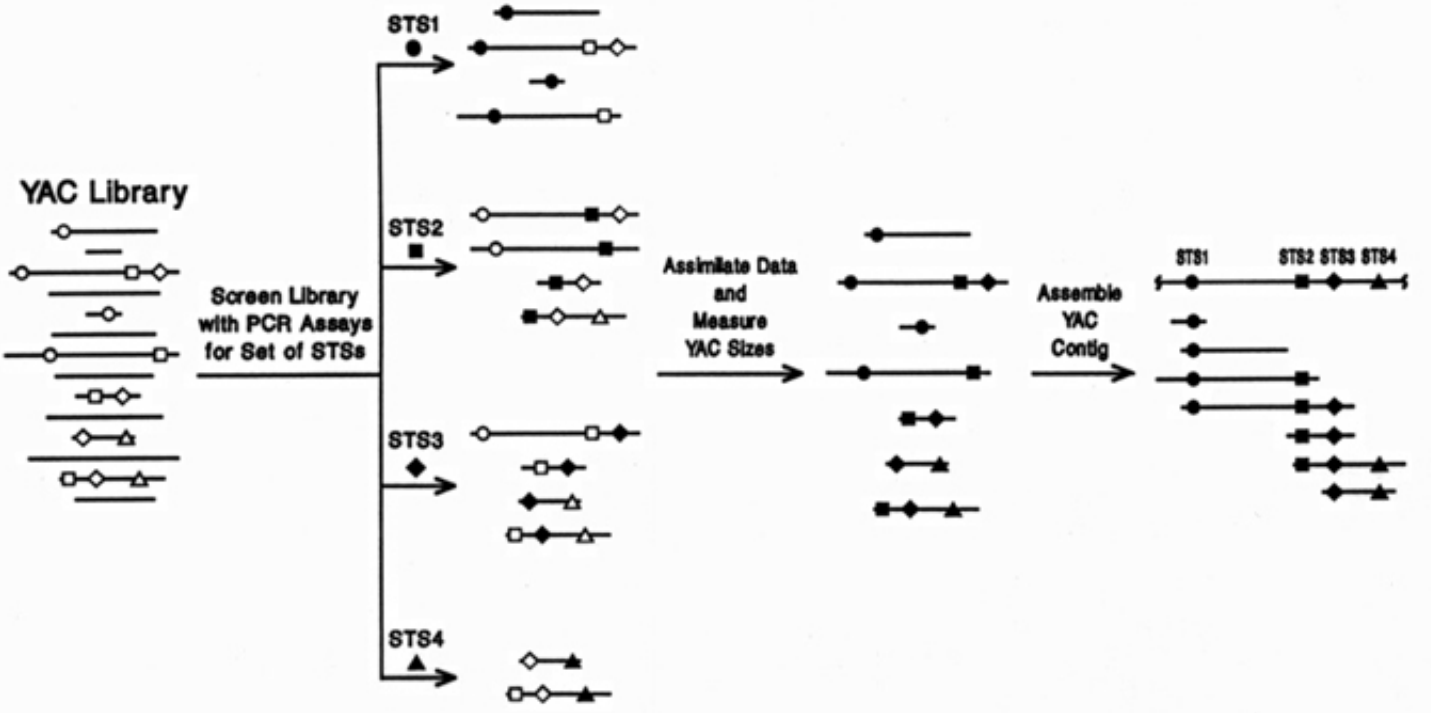


- "Common Language" Proposal by Olson et al. (1989)

A Common Language for Physical Mapping of the Human Genome

MAYNARD OLSON, LEROY HOOD,
CHARLES CANTOR, DAVID BOTSTEIN

YAC-Based STS-Content Mapping



- Green and Olson (1990) as Successful Demonstration of Paradigm

Chromosomal Region of the Cystic Fibrosis Gene in Yeast Artificial Chromosomes: A Model for Human Genome Mapping

ERIC D. GREEN AND MAYNARD V. OLSON

Implications of STSs as Landmarks for Physical Maps

- STSs are Extrinsic to the Clones

- Advantages of STSs as Landmarks

  PCR-Based (Sensitivity, Specificity, Automation)
  Electronic-Based 'Transfer' of STSs
  Landmarks Are Independent of the Mapping Resource
  Sequence-Based Nature Facilitates Integration with Sequence Map

- General Review on STS-Content Mapping: Green and Green (1991)

- Programmatic Goal of U.S. Human Genome Project

  100-kb Average Resolution STS Map of Human Genome
  [Collins and Galas (1993)]
  Therefore, ~30,000 STSs for Human Genome

- YAC-Based STS Map as 'Intermediate Map' En Route to Sequencing

Development of STSs

- Operational Definition of an STS

    1. Sequence That Can be Amplified by a PCR Assay
    2. Functionally is 'Unique' in the Genome

- DNA Sequence → Select Primers → Confirm Above Definition

- Generation of Sequences for Developing STSs (see Vollrath 1998)

    1. Non-Targeted (i.e., Genome Wide)
    2. Targeted

- Targeted Approaches

    Specific Chromosomes
        Somatic Hybrid Cell Lines (Subcloning, PCR)
        Flow Sorting
        Microdissection

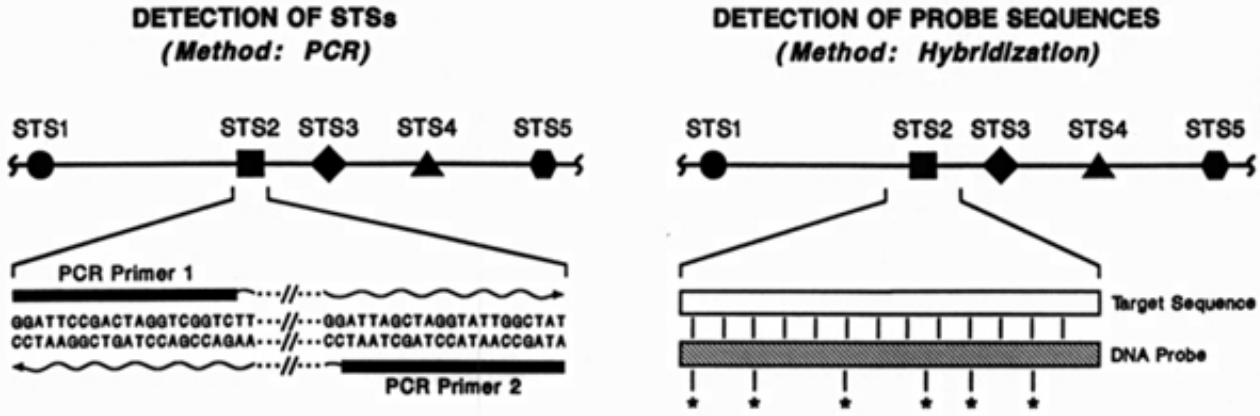
    Genetic Markers (Microsatelites)

    Expressed Sequences (Genes, ESTs)

        Note: Sequences from 3' Ends of Genes Preferred for STS Development (Since Less Likely to Contain an Intron or to be Present in Other Related Genes)

Conceptual Similarity of STSs and Probes

- Detection of STS- vs. Probe-Based Landmarks

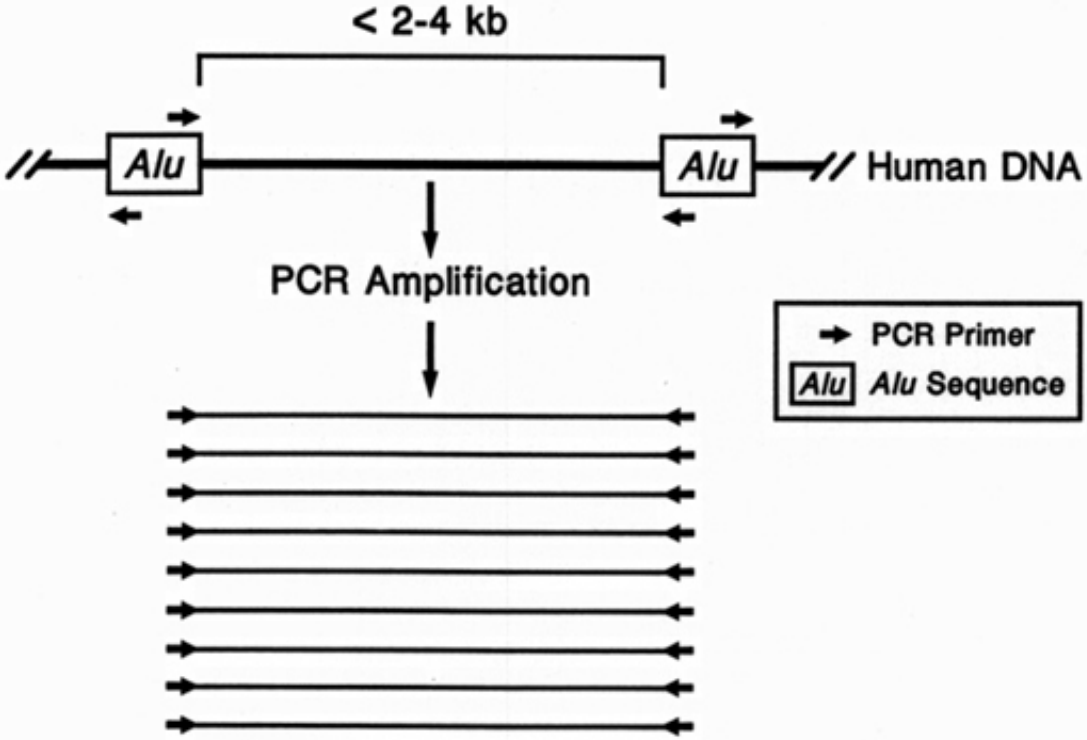


- Continued Interest in Probe-Based Map Construction

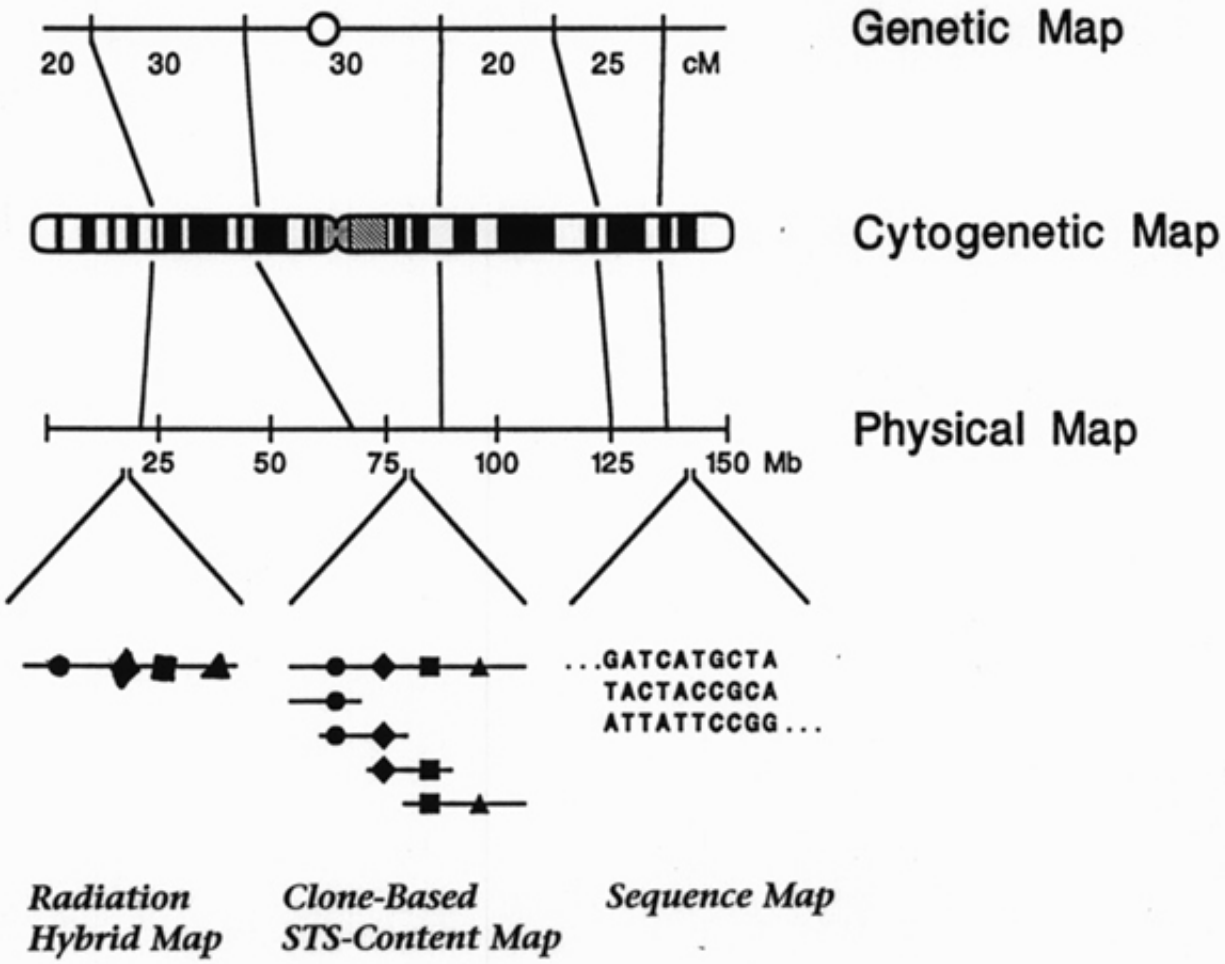
Interspersed Repetitive Sequence (IRS) PCR

- Near Ubiquitous Presence of Repetitive Sequences in Mammalian DNA
- Amplification from Priming Sites within Closely-Spaced Repeat Units
- Primers Designed to "Highly Conserved" Regions of Repeat Units
- Most Extensively Utilized IRS-PCR Method: "Alu PCR"

Nelson et al. (1989), Nelson et al. (1991), Nelson (1991)



Integration of Genomic Maps



- Importance of Map Integration

  Enhances Utility of Each Map
  Facilitates the Construction of Maps (Especially Physical Map)

- Strategies for Map Integration

  Physical → Cytogenetic
  Genetic → Physical
  (∴ Genetic → Cytogenetic)

- Importance for Both Genome-Wide and Chromosome-Specific Efforts